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(54) Title: HEMATOPOIETIC ARABINOGALACTAN COMPOSITION

(57) Abstract: Purified arabinogalactan compositions isolated from *Astragalus membranaceus*, especially from the roots of *Astragalus membranaceus* , and arabinogalactan protein compositions having a weight average molecular weight of at least 100 kiloDaltons isolated from these purified arabinogalactan compositions, are capable of reconstitution into aqueous intravenously injectable formulations; and are useful for stimulating hematopoiesis; inducing the proliferation or maturation of megakaryocytes, stimulating the production of IL-1β, IL-6, TNF-α, IFN-γ, GM-CSF, or G-CSF, stimulating the production or action of neutrophils, treating neutropenia, anemia, or thrombocytopenia, accelerating recovery from exposure (e.g. accidental or non-therapeutic exposure, as well as therapeutic exposure) to cytotoxic agents or radiation, treating cachexia, emesis, or drug withdrawal symptoms, or modifying biological responses or protecting hepatic cells in hepatitis B, in a mammal when intravenously administered to the mammal.

HEMATOPOIETIC ARABINOGALACTAN COMPOSITION

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to arabinogalactans. In particular, this invention relates to purified arabinogalactan compositions isolated from *Astragalus membranaceus*, especially from the roots of *Astragalus membranaceus* and to arabinogalactan protein compositions having a weight average molecular weight of at least 100 kiloDaltons isolated from these purified arabinogalactan compositions or intermediates thereto.

Description of Related Art

Huang-qi, Radix Astragali, is the dried root of *Astragalus membranaceus* Bge. var. *mongholicus* (Bge.) Hsiao or *A. membranaceus* (Fisch.) Bge. (Fabaceae). Huang-qi is a very old and well known drug in traditional Chinese medicine. It is officially listed in the Chinese Pharmacopoeia and used mainly as a tonic and for treatment of nephritis and diabetes. It is commonly used as a decoction or "tea" alone or with other plants in the traditional medicines Shi-ka-ron (a combination with herbs *Lithospermum erythrorhizon* and *Ligusticum wallachii*) and Ren-shen-yang-rong-tang (a combination of twelve herbs including Radix Astragali) [The section entitled "*Astragalus membranaceus* (Fisch.) Bge.", Section 26, pages 191-197, of "Chinese Drugs of Plant Origin", W. Tang and G. Eisenbrand, eds., Springer Verlag, Berlin, 1992].

Huang-qi decoctions, and solutions prepared from the alcohol-precipitated decoction, have also been administered by injection, and are reported to give improvement in the symptoms of gastric and duodenal ulcers and increase the white blood cell count in chronic leukopenia [The section entitled "Huangqi", pages 1041-1046, of "Pharmacology and Applications of Chinese Materia Medica", H.-M. Chang and P. P.-H. But, eds., World Scientific Publishing Co., Singapore, 1987]. Huang-qi decoctions, purified low molecular weight fractions (e.g. 25,000-35,000 MW), and decoctions of herb mixtures containing huang-qi, have also shown activity in restoring the immune system in local xenogeneic graft-versus-host reaction [D.-T. Chu et al., "Immunotherapy with Chinese medicinal herbs. I. . . . ", *J. Clin. Lab. Immunol.*, 25, 119-123 (1988)], reversing cyclophosphamide-induced immune suppression

[D.-T. Chu et al., "Immunotherapy with Chinese medicinal herbs. II. . . . ", *J. Clin. Lab. Immunol.*, 25, 125-129 (1988)], potentiating LAK cell cytotoxicity generated by rIL-2 [D.-T. Chu et al., "Fractionated extract of Astragalus membranaceus . ", *J. Clin. Lab. Immunol.*, 25, 183-187 (1988)], enhancing the immune response in immunodepressed mice [K. S. Zhao et al., "Enhancement of the immune response in mice by *Astragalus membranaceus* extracts", *Immunopharmacology*, 20, 225-234 (1990)], stimulating responses in mononuclear cells [Y. Sun et al., "Preliminary observations on the effects of the Chinese medicinal herbs . . . ", *J. Biol. Response Modifiers*, 2, 227-237 (1983)], and stimulating bone marrow hematopoiesis in mice [M. Rou et al., "The effect of radix astragali on mouse marrow hemopoiesis", *J. Trad. Chin. Med.*, 3(3), 199-204 (1983); S. I. Miura et al., "Effect of a traditional Chinese herbal medicine . . . ", *Int. J. Immunopharmacol.*, 7(11), 771-780 (1989); and Y. Ohnishi et al., "Effects of Juzen-taiho-toh (TJ-48) . . . ", *Exp. Hematol.*, 18, 18-22 (1990)].

Liu, US Patent No. 4,843,067, discloses a pharmaceutical composition containing polysaccharides of huang-qi (stated to be extractable from either *Astragalus membranaceus* Bge. or *Astragalus gummifer* Labillard) and polysaccharides of dankuei. The huang-qi polysaccharides are stated to be extractable by water extraction of a powder of the roots and ethanol precipitation. Verbiscar, US Patent No. 5,2868,467, discloses immunomodulatory polysaccharide fractions from the plants of *Astragalus tragacantha* (tragacanth), prepared at low temperature to "maintain the integrity of the polysaccharide toward chemical and conformational changes". Josephson et al., US Patent No. 5,336,506, discloses the use of plant polysaccharides such as arabinogalactans (isolated from larch, *Larix occidentalis*) and mannans to form complexes with therapeutic agents for the targeting of the therapeutic agent to a cell receptor capable of receptor-mediated endocytosis. Adams et al., US Patent No. 5,116,969, discloses an ultrarefined arabinogalactan product said to be suitable for use in density gradient separation. Jung et al., US Patent No. 5,478,576, discloses purified arabinogalactans (also from *Larix occidentalis*), degradative products, and modifications thereof, also for use in delivering therapeutic agents to cell receptors capable of receptor-mediated endocytosis. Lewis, US Patent No. 5,589,591, discloses endotoxin-free polysaccharides, such as arabinogalactans, dextrans, mannans, and gum arabic, prepared from impure

forms of these polysaccharides by ultrafiltration through first through a low molecular weight cutoff membrane, keeping the retentate, and then through a high molecular weight cutoff membrane, keeping the filtrate.

Each of these references, however, devotes its attention to the polysaccharides (e.g. arabinogalactans) present in the products, and may even use techniques designed to exclude arabinogalactan proteins.

Arabinogalactan proteins are also found in flowering plants, and are widely distributed in most higher plants. Arabinogalactan proteins (AGPs), sometimes referred to as arabinogalactan peptides, are glycosylated proteins containing high proportions of carbohydrate and usually a low (less than 10%) protein content, although AGPs having a higher protein content are known. Among the hydroxyproline-rich glycoproteins isolatable from plants, AGPs are characterized by their generally low protein content and their general ability to bind the β-glucosyl Yariv reagent, 1,3,5-tris(4-β-D-gluco-pyranosyloxyphenylazo)-2,4,6-trihydroxybenzene, [J. H. Yariv et al., *Biochem. J.*, **85**, 383-388 (1962); R. L. Anderson et al., *Aust. J. Plant Physiol.*, 4, 143-158 (1977)]. AGPs are components of gum arabic, a gummy exudate from the acacia tree, *Acacia senegal*, that is frequently used in food products as an emulsifier, crystallization preventer, and flavor encapsulator. The isolation of plant AGP genes from *Nicotiana alata*, *Nicotiana plumbaginafolia*, and *Pyrus communis* is disclosed in Chen et al., US Patent No. 5,646,029. An extensive discussion of AGPs may be found in E. A. Nothnagel, "Proteoglycans and related components in plant cells", *Int. Rev. Cytology*, **174**, 195-291 (1997).

The disclosures of these and other documents referred to throughout this application are incorporated herein by reference.

SUMMARY OF THE INVENTION

In a first aspect, this invention provides a purified arabinogalactan composition isolated from *Astragalus membranaceus*, especially from the roots of *Astragalus membranaceus*.

In a second aspect, this invention provides an arabinogalactan protein composition having a weight average molecular weight of at least 100 kiloDaltons isolated from the purified arabinogalactan composition of the first aspect of this invention.

In a third aspect, this invention provides an aqueous intravenously injectable arabinogalactan formulation comprising a therapeutically effective amount of the purified arabinogalactan composition of the first aspect of this invention or the arabinogalactan protein composition of the second aspect of this invention, and an aqueous intravenously injectable excipient.

In a fourth aspect, this invention provides a method of treating a disease state in a mammal capable of treatment by administration of the purified arabinogalactan composition of the first aspect of this invention or the arabinogalactan protein composition of the second aspect of this invention (such as stimulating hematopoiesis, inducing the proliferation or maturation of megakaryocytes, stimulating the production of IL-1β, IL-6, TNF-α, IFN-γ, GM-CSF, or G-CSF, stimulating the production or action of neutrophils, treating neutropenia, anemia, or thrombocytopenia, accelerating recovery from exposure (e.g. accidental or non-therapeutic exposure, as well as therapeutic exposure) to cytotoxic agents or radiation, treating cachexia, emesis, or drug withdrawal symptoms, or modifying biological responses or protecting hepatic cells in hepatitis B, in a mammal), comprising intravenously administering to the mammal an effective amount of the purified arabinogalactan composition of the first aspect of this invention or the arabinogalactan protein composition of the second aspect of this invention, especially as an aqueous intravenously injectable arabinogalactan formulation of the third aspect of this invention; optionally in conjunction with at least one other therapeutic agent (such as one capable of stimulating hematopoiesis).

In a fifth aspect, this invention provides methods of preparing the purified arabinogalactan composition of the first aspect of this invention, the arabinogalactan protein composition of the second aspect of this invention, and the aqueous intravenously injectable arabinogalactan formulation of the third aspect of this invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the mean white blood cell counts versus days after chemotherapy for human cancer patients receiving chemotherapy followed by no treatment, treatment with a purified arabinogalactan composition of this invention, or treatment with G-CSF.

FIG. 2 shows the total symptom scores versus days after chemotherapy for human cancer patients receiving chemotherapy followed by no treatment, treatment with a purified arabinogalactan composition of this invention, or treatment with G-CSF.

FIG. 3 shows the scores on the Karnofsky Performance Index for human cancer patients receiving chemotherapy followed by no treatment, treatment with a purified arabinogalactan composition of this invention, or treatment with G-CSF.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions

An "arabinogalactan protein" or "AGP" is a generally β-glucosyl Yariv reagent-precipitable, highly glycosylated protein in which the carbohydrate accounts for at least 50% by weight of the molecule, and in which the major carbohydrate constituents are arabinose and galactose, with the arabinosyl residues primarily in terminal positions. It generally reacts specifically with a monoclonal antibody, MAC207, for AGPs.

An "arabinogalactan protein composition" is a composition comprising at least 70%, particularly at least 80%, more particularly at least 90%, by weight of the composition of arabinogalactan protein (as defined above) and associated arabinogalactans and other polysaccharides.

A "purified arabinogalactan composition" is a composition comprising an arabinogalactan protein (as defined above) and associated arabinogalactans and other polysaccharides.

"Mammal" includes humans and non-human mammals, such as companion animals (cats, dogs, and the like) and farm animals (cattle, horses, sheep, goats, swine, and the like).

"Disease" includes any unhealthy condition of an animal, including an unhealthy condition resulting from medical therapy (a "side-effect"), such as disease states in which a blood tonifying effect is therapeutic, including particularly those disease states listed in the "Pharmacology and Utility" section of this application.

"Pharmaceutically acceptable excipient" means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for veterinary use as well as for human pharmaceutical use. Such excipients may be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous.

A "therapeutically effective amount" means the amount that, when administered to an animal for treating a disease, is sufficient to effect treatment for that disease.

"Treating" or "treatment" of a disease includes preventing the disease from occurring in an animal that may be predisposed to the disease but does not yet experience or exhibit symptoms of the disease (prophylactic treatment), inhibiting the disease (slowing or arresting its development), providing relief from the symptoms or side-effects of the disease (including palliative treatment), and relieving the disease (causing regression of the disease).

The Purified Arabinogalactan Composition

The purified arabinogalactan composition of this invention is a "purified arabinogalactan composition" as that term is defined above that is isolated from *Astragalus membranaceus*, especially from the roots of *Astragalus membranaceus*; preferably from the roots of *A. membranaceus* Bge. var. *mongholicus* (Bge.) Hsiao or *A. membranaceus* (Fisch.) Bge.; preferably where the roots are from *A. membranaceus* plants grown in Inner Mongolia or Shanxi province, Peoples' Republic of China, especially the former; and preferably where the roots are from two-year-old *A. membranaceus* plants. It has a typical sugar composition (determined by GLC of the trimethylsilyl derivatives of the methanolyzed composition) containing about between 5 and 15 mole %, particularly about 10%, Ara; less than about 1.5%, particularly less than about 1%, Rha; up to about 4% GalA; about between 3 and 7% Gal; and about between

70 and 90% Glc; with an Ara:Gal ratio of not less than 1.5:1, particularly not less than about 1.75:1, and typically less than about 3:1; an ash content of not more than about 2% by weight; a heavy metal content of not more than about 10 ppm by weight; and a hydroxyproline content of not more than about 0.1%, particularly not more than about 0.05%. It is substantially free of endotoxin (i.e. having an endotoxin content, as determined by Endospecy [Seikagaku Corporation, Tokyo, Japan] assay following the manufacturer's instructions, of less than 1.0 EU/mg, particularly less than 0.8 EU/mg, more particularly less than 0.5 EU/mg, especially less than 0.3 EU/mg); soluble in water to at least 20 mg/mL, having a pH in aqueous solution between 4.5 and 6.5; and has a weight average molecular weight (Mw) between 20 and 60 kiloDaltons, particularly between 25 and 40 kiloDaltons, and more particularly between 27 and 35 kiloDaltons. For convenience, this material may be referred to further as "PAGC".

Preparation of the Purified Arabinogalactan Composition

The purified arabinogalactan composition is prepared by extracting the *Astragalus membranaceus* (typically the sterile processed chipped or sectioned dried roots, prepared by trimming the dried roots, scrubbing with ultrafiltered (UF) water, cleaning with a disinfecting solution such as 70% ethanol, cutting into thin slices, and drying under sterile conditions), referred to as "drink chips", with hot water (typically at not less than 80 °C, particularly not less than 90 °C, especially at about 100 °C), optionally in the presence of a co-extractant such as an alkali metal salt, especially potassium or sodium dihydrogen phosphate, for a time, at a temperature, and for as many extraction cycles are necessary or desirable to cause substantial extraction of the arabinogalactan protein and associated polysaccharides from the roots (typically three times each for 3 hours at 100 °C). All steps following the preparation of the dried comminuted roots are typically conducted under aseptic conditions employing sterile equipment and reagents. The hot water extract is concentrated (typically under vacuum at 60 – 70 °C to a concentration of about 1L/Kg of "drink chips"), and then precipitated with a lower alkanol (such as ethanol, at a final ethanol concentration of about 70% at about room temperature). The lower alkanol precipitate is typically washed with further lower alkanol (typically three times with 95% ethanol) and then suspended with water at a suitable concentration for further processing (typically 18 – 20% weight/volume). The

aqueous suspension is then treated to remove materials that are not water-soluble, such as by re-precipitating with a lower alkanol (e.g. ethanol at an ethanol concentration of about 35%). The supernatant of the lower alkanol precipitation, for example 35% ethanol precipitation, is further precipitated with a higher concentration of lower alkanol, for example 40 – 80% ethanol, particularly 60-70% ethanol, to precipitate a crude arabinogalactan composition containing the arabinogalactan protein and associated polysaccharides. The precipitate is re-dissolved in water and may be dried (typically by spray drying, to avoid excessive heating) to isolate the crude arabinogalactan composition. The crude arabinogalactan composition will typically be a light yellow powder, soluble in water to at least about 100 mg/mL, particularly at least about 200 mg/mL, with a weight loss on drying of less than about 15% and having an endotoxin content of less than 0.5, particularly less than 0.3 EU/mg.

To accommodate batch-to-batch variations in the *Astragalus membranaceus* raw material, blending of the raw material, the "drink chips", and intermediates in the process may be used to achieve consistency of final product.

The crude arabinogalactan composition is then purified by ion-exchange chromatography. It is dissolved in water, or the re-dissolved aqueous solution of the precipitate is brought, to a suitable concentration (typically about 2%) and then ultrafiltered to further remove low molecular weight materials and to reduce the volume of the solution (such as by using a 5 kiloDalton molecular weight cutoff (5K MWCO) ultrafiltration (UF) system). The retentate obtained from the ultrafiltration is then eluted through a cation exchange column (such as a SP Sepharose cation exchange column equilibrated with 20 m*M* NaOAc buffer, pH 5.20); and the eluate loaded onto and eluted through an anion exchange column (such as a Q Sepharose anion exchange column equilibrated with the same NaOAc buffer). The eluate from the anion exchange column may be used directly in the preparation of the arabinogalactan protein composition of the second aspect of this invention, may be concentrated and dried to form an intermediate suitable for preparation of the arabinogalactan protein composition, or may be used directly in the preparation of the purified arabinogalactan composition. In the preparation

of the purified arabinogalactan composition, it may be microfiltered through a suitable bacteriostatic filter (such as a 0.1 μm filter), and is ultrafiltered to desalt the solution and again reduce its volume (such as by an 8K MWCO UF system). The retentate from the ultrafiltration is concentrated (such as to about 20 – 26% at 50 – 60 °C), and then precipitated with a lower alkanol (such as with ethanol at a concentration of about 80 - 90%). The precipitate may be further washed (such as with anhydrous ethanol, three times), and then dried (such as in a vacuum oven at 60 – 70 °C) to give the purified arabinogalactan composition.

The arabinogalactan protein composition

The arabinogalactan protein composition of this invention is an "arabinogalactan protein composition" as that term is defined above that is isolated from *Astragalus membranaceus*, especially from the roots of *Astragalus membranaceus*; preferably from the roots of *A. membranaceus* Bge. var. *mongholicus* (Bge.) Hsiao or *A. membranaceus* (Fisch.) Bge.; preferably where the roots are from *A. membranaceus* plants grown in Inner Mongolia or Shanxi province, Peoples' Republic of China, especially the former; and preferably where the roots are from two-year-old *A. membranaceus* plants. It has a typical sugar composition containing about between 45 and 75 mole %, particularly about between 50 and 75%, Ara; about between 2 and 4%, Rha; about between 4 and 6% GalA; about between 8 and 25%, particularly about between 10 and 20% Gal; and about between 5 and 25% Glc; with an Ara:Gal ratio of not less than 2:1, typically not less than about 3:1, and especially not less than about 4:1; an ash content of not more than about 2% by weight; a heavy metal content of not more than about 10 ppm by weight; and a hydroxyproline content of not less than about 0.2%, particularly not less than about 0.3%. It is substantially free of endotoxin, as that term is defined above; soluble in water to at least 20 mg/mL, having a pH in aqueous solution between 4.5 and 6.5; and has an Mw (as defined above) of not less than 100 kiloDaltons, particularly between 150 and 350 kiloDaltons. It contains about 95% carbohydrates (including the carbohydrates that glycosylate the protein core of the arabinogalactan protein) and about 5% proteins. Hydroxyproline, accounting for about 20% of its total amino acid content, is a characteristic of the arabinogalactan protein. For convenience, this material may be referred to further as "AGPC".

Preparation of the arabinogalactan protein composition

The arabinogalactan protein composition may be prepared from the purified arabinogalactan composition described above or from the eluate from the ion-exchange purification step (or a solid intermediate prepared by concentrating and drying that eluate), by ultrafiltration with a 100K MWCO UF system. Typically, the eluate from the anion exchange column described in the "Preparation of the purified arabinogalactan composition" above is applied directly to the 100K MWCO UF system. The retentate from this 100K ultrafiltration is then further concentrated, precipitated with a lower alkanol, optionally further washed, and dried, all in a manner similar to that described above for the preparation of the purified arabinogalactan composition, to give the arabinogalactan protein composition.

Pharmacology and utility

The beneficial activity of the purified arabinogalactan composition of the first aspect of this invention or the arabinogalactan protein composition of the second aspect of this invention have been shown in several tests, leading to the following utilities.

Treatment of neutropenia in patients undergoing chemotherapy

1. Production of cytokines from activated human peripheral blood mononuclear cells (PBMC)

Since stimulation of the immune and hematopoietic system occurs via multiple cytokine interactions, the ability of PAGC to induce the production of cytokines *in vitro* was examined as described in Example 3. PAGC triggered significant, dose-dependent release of IL-1β, IL-6, TNF-α, IFN-γ, GM-CSF and G-CSF by human PBMC after activation with PHA. The three cytokines, IL-6, GM-CSF and G-CSF, are known to affect the production/or action of neutrophils *in vitro* and *in vivo*. IL-6, by itself or in combination with other cytokines, has been reported to stimulate bone marrow megakaryocyte maturation and platelet recovery in peripheral blood in mice and nonhuman primates. These data suggest that PAGC may stimulate production of

neutrophils and recovery of platelet counts in myelosuppressed patients via the production of cytokines involved in hematopoietic functions.

2. Recovery of GM-CFC progenitor cells in fluorouracil-treated mice

PAGC has been tested in a short-term, murine, *ex vivo* model of hematopoietic progenitor recovery as described in Example 5. Fluorouracil has been widely used in mice to deplete bone marrow progenitors, colony forming units in culture (CFU-C). Because quiescent stem cells are unaffected by fluorouracil, the mice recover from this treatment along a predictable, well-documented time course [A. M. Yeager et al., "The effects of 5-fluorouracil on hematopoiesis: studies of murine megakaryocyte-CFC, granulocyte-macrophage-CFC, and peripheral blood cell levels", *Exp. Hematol.*, 11, 944-952 (1983)]. PAGC increased, in a dose-dependent manner, the number of GM-CFC per femur on day 4 after fluorouracil ablation as can be seen from the table in Example 5. When PAGC was administered at 200 mg/Kg, the mean increase in GM-CFC per femur was 3.3-fold greater than controls ($p<0.01$). Animals treated with PAGC at 100 mg/Kg displayed a mean GM-CFC per femur that was 1.8-fold greater than the control, and animals treated with 50 mg/Kg PAGC displayed a mean GM-CFC similar to the control. These data show that PAGC enhances bone marrow progenitor (GM-CFC) recovery from fluorouracil-induced myelosuppression in mice; and may explain, at least in part, the mechanism through which PAGC enhances the recovery of peripheral white blood cell counts in myelosuppressed mice.

3. Recovery of peripheral white blood cells in sublethally irradiated mice

BALB/c mice were sublethally irradiated and then treated subcutaneously with saline or various doses of PAGC according to the protocol described in Example 6. Irradiation dramatically decreased the number of WBC to 12% of normal on day 14 post-irradiation. Treatment with PAGC increased the total WBC counts to values above those seen in the saline-treated control animals. Groups treated with 100 or 300 mg/Kg of PAGC recovered WBC counts to 80% of normal (normal range: 6000-10000 WBC/μL blood) approximately 7-9 days ahead of the saline-treated group. In the PAGC-treated animals, normal WBC counts were reached by days 22-23, when saline-treated controls

had only about 46% of normal WBC counts. Additionally, differential WBC counts were determined using stained peripheral blood smears. Using this information, absolute neutrophil counts and absolute lymphocyte counts were calculated. Treatment with PAGC increased the absolute neutrophil count to values above those seen in the saline-treated control animals, and also increased absolute lymphocyte counts above control values, in this model.

4. Restoration of white blood cell counts after chemotherapy

A phase II clinical trial has been completed in the Peoples' Republic of China. A subpopulation of patients whose WBC counts were less than 3.0×10^9 WBC/L at the time of entering to the study were analyzed: of these, 168 patients were in the PAGC, 59 in the G-CSF and 23 in the no-treatment group. The treatment protocol is discussed in Example 9. As can be seen from FIG. 1, PAGC treatment steadily increased the WBC counts of patients after chemotherapy and this increase continued to day 14. Although administration of G-CSF showed more rapid increase of the WBC count to a maximum on day 7, this increase was not sustained and started to drop after day 7. Both treatment groups achieved normal WBC earlier than the no treatment group. The PAGC group achieved statistical significance when compared with the no-treatment group on day 10 and day 14, but there was no statistical difference in the WBC count between the PAGC group and the G-CSF group on day 14. The above results indicate that the use of PAGC as an adjunct to chemotherapy in patients with lung, gastroenteric, and breast cancer was safe and well tolerated. Treatment with PAGC restored patients' WBC count after chemotherapy. No clinical significant adverse events were observed from the administration of PAGC.

Treatment of thrombocytopenia in patients undergoing chemotherapy

1. Recovery of peripheral blood platelet counts in sublethally irradiated mice

BALB/c mice were sublethally irradiated on day 0 and then treated subcutaneously with saline or PAGC, as described in Example 6. Irradiation dramatically decreased the number of platelets to 7% of normal on day 10 post-irradiation. Subcutaneous PAGC treatment at 100 or 300 mg/Kg significantly enhanced platelet

recovery in the peripheral blood of the mice. PAGC induced recovery of platelet counts to 80% of normal, which ranged between 8-12 $x10^5$ platelets/μL blood, about 5-6 days earlier than the saline control group. Treatment with PAGC resulted in an increase in platelet counts to normal levels by days 24-25 post-irradiation, whereas saline-treated controls had only recovered to 72% of normal within the same period. These findings suggest that PAGC is a highly efficacious agent for enhancing platelet development and should be considered useful for the treatment of thrombocytopenia. AGPC administered in the same manner in a 20-day study at doses of 100 and 250 mg/Kg promoted both red blood cell and platelet recovery with improvement over control in irradiated mice at all points post-irradiation; demonstrating the same benefit in this model as that given by PAGC.

2. Proliferation/maturation of bone marrow megakaryocytes

PAGC accelerated the recovery of the peripheral blood platelet count in an animal model of irradiation induced bone marrow suppression as shown in the preceding paragraph, suggesting that PAGC could act by stimulating the proliferation and/or maturation of bone marrow megakaryocytes. This possibility was investigated using an *in vitro* liquid culture system as described in Example 4. From a dose titration curve, the optimal dose of PAGC in this model was 100-200 μg/mL, with an ED_{50} of 30 - 40 μg/mL. This study demonstrated that PAGC alone could promote the proliferation and/or maturation of bone marrow megakaryocytes *in vitro*. Also, PAGC acted synergistically with a suboptimal dose of IL-3 (50 pg/mL) to increase acetylcholinesterase (AchE) levels in a dose-dependent manner. The amount of AChE obtained in cultures treated with 200 μg/ml of PAGC and 50 pg/ml of IL-3 was equivalent to the level obtained with the optimal dose of IL-3. These data suggest that PAGC can enhance the responsiveness of megakaryocytes to much lower concentrations of IL-3, or alternatively IL-3 could increase the responsiveness of megakaryocytes to PAGC. Since chemotherapy or radiation therapy will damage many cells and tissues including cytokine-producing cells, patients so treated may have lower levels of endogenous cytokine secretion. Such low levels of endogenous cytokines may not support the normal functions of the hematopoietic system, which therefore cannot generate adequate numbers of hematopoietic cells for recovery from bone marrow

suppression. Therefore, PAGC may be a good candidate to remedy this situation. The results from this study demonstrate that PAGC can enhance the proliferation/maturation of bone marrow megakaryocytes to a normal level in the presence of otherwise insufficient levels of endogenous cytokines. As shown above, PAGC stimulates the recovery of peripheral blood platelets and seems to do so by inducing the proliferation and/or the maturation of megakaryocytes. The data suggest that PAGC may prove useful in the treatment of thrombocytopenia that occurs secondary to bone marrow suppression.

3. Increase of platelet counts after chemotherapy

A Phase II clinical trial is described in Example 9. A subpopulation of patients had WBC count less than 4.0×10^9/L and platelet counts less than 90×10^9/L at the time of entering the study. There were 54 such patients in the PAGC group. These patients' platelet counts increased to greater than 100×10^9/L on day 7 and the counts continued to increase through day 14 as seen in the table in Example 9. These data show that PAGC increased the platelet counts of these patients after chemotherapy.

Improvement of quality-of-life of cancer patients

A Phase II clinical trial is described in Example 9. The quality-of-life was assessed on all the patients entered to the study by improvement on symptoms associated with chemotherapy and Karnofsky Performance Index score as described in Example 9. Chemotherapy-induced symptoms, including lassitude and fatigue, malaise, sweating, shortness of breath and lack of appetite, were scored and totaled as the symptom score during the treatment period. As illustrated in FIG. 2, as the treatment progressed, the PAGC group demonstrated a more rapid recovery from these symptoms as indicated by the decreased score and approached normal (score = 0) faster than the G-CSF group. Additionally, the PAGC group showed a statistical significant improvement in the score compared with G-CSF group on day 10 through day 14 with $p<0.01$. However, there was no statistical difference between the G-CSF and the no-treatment groups on the score on all the 14 days monitored. These data suggested that PAGC can improve patients' symptoms associated with chemotherapy and that G-CSF, however, does not seem to help patients in this direction. A subpopulation of patients with Karnofsky scores less

than 70 before the treatment were analyzed for the improvement on their scores after various treatments. The PAGC group demonstrated a statistically significant improvement over the G-CSF and no-treatment groups, with $p<0.01$ and $p<0.0001$ respectively, as shown in FIG. 3. More of the patients in the PAGC group had higher score than the other two groups.

Prevention of neutropenia in cancer patients undergoing chemotherapy

In a prior section, PAGC was shown effective for treatment of chemotherapy-induced neutropenia. The data suggest also that PAGC may be useful in the prevention of neutropenia as well as in the treatment of neutropenia, and clinical trials are planned in the PRC for this indication.

Treatment of neutropenia in cancer patients undergoing radiation therapy

The pharmacology data given previously also suggest the use of PAGC for the recovery of neutropenia in radiation therapy. A clinical trial has been planned in the PRC to look at the treatment of neutropenia in patients with non-Hodgkin's lymphoma after radiation therapy.

Treatment of anemia in cancer patients undergoing chemotherapy

BALB/c mice were sublethally irradiated (4.25 Gy) and then treated with saline or various doses of PAGC according to the protocol described in Example 6. Irradiation dramatically decreased the number of RBC to 55% of normal on day 17 post-irradiation. PAGC treatment resulted in significantly higher RBC counts on day 17, when RBC counts of saline-treated control animals were still at their lowest point. In animals given 100 or 300 mg/Kg of PAGC, RBC counts rose to 80% of normal ($8.5 - 11 \times 10^6/\mu L$ blood) approximately 4-6 days ahead of the saline-treated group. These PAGC-treated animals showed RBC counts in the normal range by days 20-22, whereas, on those same days, the saline-treated controls had only about 65% of normal RBC counts. These results indicate that PAGC may affect erythroid progenitor cell development and/or mobilization, suggesting its usefulness in radiotherapy- and chemotherapy-induced anemia. AGPC administered in the same manner in a 20-day study at doses of 100 and

250 mg/Kg promoted both red blood cell and platelet recovery with improvement over control in irradiated mice at all points post-irradiation; demonstrating the same benefit in this model as that given by PAGC.

The phase II trial of Example 9 was targeted to study the recovery of WBC counts in patients after chemotherapy, and the inclusion criteria were based on WBC counts only. An additional clinical trial has been planned in the PRC to study the use of PAGC for the treatment of anemia, targeting patients with anemia after chemotherapy.

Mobilization of peripheral blood progenitor cells alone or in combination with G-CSF for patients undergoing peripheral blood progenitor cell transplantation

The CD34 antigen is present on hematopoietic stem cells and progenitor cells, including colony-forming cells such as BFU-E (burst-forming unit – erythroid), GM-CFC (granulocyte macrophage colony forming cells), and CFU-Mix (colony forming unit – mixture) cells. The assay by flow cytometry of $CD34^+$ cells provides a convenient determination of graft composition. The existence of hematopoietic progenitor cells in the peripheral blood of humans was first described in 1975. These peripheral blood progenitor cells (PBPC) make up only a small fraction of the total number, since the vast majority of progenitors reside in the bone marrow. Increases in the numbers of peripheral blood progenitor cells in humans during the rebound following chemotherapy were reported in 1976. More recently, the colony stimulating factors G-CSF and GM-CSF have been demonstrated to directly augment the numbers of PBPC. The combination of chemotherapy and colony stimulating factors greatly increases the numbers of PBPC above increases observed with either method alone. Currently, G-CSF is used in humans to mobilize PBPC for autologous and allogeneic transplantation. Transplantation of PBPC leads to more rapid engraftment than conventional bone marrow transplantation. The yield of PBPC generated with G-CSF treatment can be greatly enhanced when used in combination either with chemotherapy, as mentioned above, or with other cytokines. The ability of PAGC to induce PBPC mobilization when administered as a single agent and/or to synergize with G-CSF to induce PBPC mobilization when administered in combination with G-CSF was demonstrated in Example 7. As can be seen in the table in Example 7, PAGC resulted in a 6-fold mean

increase in circulating GM-CFC. Additionally, PAGC synergized with G-CSF, resulting in an 83-fold mean increase in circulating GM-CFC. This increase was significantly better than the 39-fold increase observed with G-CSF alone. The results showed that PAGC increased the numbers of circulating GM-CFC and synergized with G-CSF to increase the numbers of circulating GM-CFC in normal mice. PAGC also resulted in a 3-fold mean increase in circulating BFU-E, as shown in the table, and synergized with G-CSF, resulting in a 9.5-fold mean increase in circulating BFU-E. This increase was significantly better than the 4.5-fold increase observed with G-CSF alone. In a similar experiment with cyclophosphamide-treated mice, an increase in the number of $CD34^{+}Lin^{-}$ cells was seen over mice not treated with PAGC. Therapeutic agents such as PAGC which synergize with G-CSF to increase the yields of PBPC will likely be very useful. This type of synergy may reduce costs by decreasing the number of aphereses necessary to harvest PBPC from donors. Additionally, this type of combination therapy may help in situations where recipients are not sufficiently responsive to G-CSF alone or where the use of chemotherapeutic drugs is not desirable. Similarly, AGPC should offer the same benefit.

Acceleration of healing from exposure to cytotoxic agents or radiation

From the results given above, where animals or human patients were deliberately exposed to radiation or cytotoxic agents and demonstrated an accelerated healing over animals or human patients not so treated, PAGC will also be useful in accelerating recovery from exposure (e.g. accidental or non-therapeutic exposure, as well as therapeutic exposure) to cytotoxic agents or radiation.

Treatment of cachexia

One of the most common side effects of chemotherapy and radiation therapy is that patients suffer from lack of appetite and weight loss. The study of Example 8 has shown that PAGC could increase the body weight of mice treated with chemotherapeutic agent cyclophosphamide or fluorouracil. The mice treated with PAGC lost less weight and regained weight faster than those treated with the chemotherapeutic agents alone, although these differences were not statistically significant.

As discussed above, PAGC has clearly demonstrated to be effective in improving patients' quality-of-life in a Phase II clinical trial. One of the parameters measured was improvement in appetite. This, together with the mouse study, suggests that PAGC could help patients with cachexia, a general physical wasting and malnutrition caused by a chronic disease such as cancer or the therapies for it.

Combination therapy with G-CSF in cancer patients undergoing myelosuppressive therapy to increase neutrophil recovery and to reduce the use of G-CSF

The studies discussed previously have demonstrated that PAGC stimulates the production of cytokines especially G-CSF from activated human peripheral blood mononuclear cells, promotes recovery of GM-CFC and peripheral WBC counts in radiation-induced myelosuppression animal model, and restores the WBC counts of cancer patients after chemotherapy in a Phase II clinical trial. These results suggest that PAGC may act indirectly on the hematopoiesis system through production of multiple endogenous cytokines and these cytokines may act in synergy to promote neutrophil recovery. PAGC is also shown to have a synergistic effect with IL-3 in promoting the proliferation/maturation of bone marrow megakaryocytes as discussed above.

In addition, the administration of PAGC was safe and well tolerated by patients in the clinical trial. The adverse reactions seen in the use of G-CSF such like bone pain, muscle ache, headache, fatigue, nausea, vomiting, diarrhea, etc. were not observed in the administration of PAGC. This, and the synergistic effects discussed above, suggest that PAGC may combine with G-CSF to reduce the use of exogenous G-CSF to promote neutrophil recovery in cancer patients undergoing myelosuppressive therapy.

Combination therapy with G-CSF to accelerate neutrophil recovery following high-dose cytotoxic therapy with autologous or allogeneic blood progenitor cell transplantation

High-dose chemotherapy and/or radiotherapy supported by (marrow or peripheral blood) blood progenitor cell (BPC) transplant is being used to treat a number of cancer patients with conditions such as breast cancer, lymphoma, and multiple myeloma. Transplantation with BPC results in more rapid hematopoietic recovery. G-CSF is often

used after BPC transplantation to accelerate neutrophil recovery in order to prevent neutropenic fever associated with infection, to shorten the hospital stay, and to reduce the use of antibiotics. PAGC can restore cancer patients' WBC counts after chemotherapy as shown in the Phase II clinical trial of Example 9; and that together with the pharmacological effects summarized in previous sections suggest that PAGC may also be useful as a combination therapy with G-CSF after BPC transplantation to accelerate neutrophil recovery.

Biological response modifier for Hepatitis B carriers and protection of hepatic cells

Cytokines play an important role in the defense against viral infections. The cytokines produced by cells involved in the immune response, such as macrophages and CD4+ and CD8+T lymphocytes, play a more direct role in recovery from viral infection such as hepatitis B viral (HBV) infection. From animal models and human studies, it is clear that the cellular immune response may play a role in resolution of HBV infection and disease pathogenesis. In acute HBV infection, a vigorous polyclonal cellular immune response is critical. Type 1 cytokine release, characterized by production of IL-2 and IFN-γ by CD4+ T cells, which prime and maintain antigen-specific cellular immunity, is important in defense against viral infection [C. A. Biron, "Cytokines in the generation of immune response to, and resolution of, virus infection", *Curr. Opin. Immunol.*, 6, 530-538 (1994)]. The cytokines released by CD4+ and CD8+ cells also play an important role in the downregulation of HBV replication. If there is a defect in the acute response, HBV becomes chronic. These finding suggest that strategies aimed at boosting the type 1 response or the local production of appropriate cytokines within the liver might be useful as therapy for chronic HBV infection [M. J. Koziel, *Sem. Liver Disease*, 19(2), 157-161 (1999)]. PAGC is prepared from the traditional Chinese medicinal plant *Astragalus membranaceus* var. *mongholicus* (AM) and this plant has been used historically to stimulate the immune and hematopoietic systems. It is widely used to treat patients with various ailments, which are similar to the symptoms of chemotherapy- or radiotherapy-induced myelosuppression (thrombocytopenia and anemia in addition to neutropenia). Additionally, AM has been reported to stimulate mouse spleen cell proliferation in a dose-dependent manner *in vitro*, increase the natural killer (NK) cell activity in spleen cells of animals inoculated with S-180 sarcoma tumor cells, and also

increase the activity of cytotoxic T lymphocytes (CTL). The cytokines produced by CTL can mediate control of viral infection *in vivo*, and the production of IFN-γ and TNF-α by virus-specific CTL can amplify the ability of CTL to clear viral infection [L. G. Guidotti et al., "Cytotoxic T lymphocytes inhibit hepatitis B virus gene expression by a noncytolytic mechanism in transgenic mice", *Proc. Natl. Acad. Sci. USA*, 91, 764-3768 (1994)]. Furthermore, as shown above, PAGC stimulated the production of cytokines IL-1β, IL-6, TNF-α, IFN-γ, GM-CSF and G-CSF from human PBMC after activation with PHA. These studies suggest that PAGC may affect immune function indirectly through modulation of cytokine production. Crude extracts prepared from AM have been used in chronic hepatitis patients to reduce the elevated IgG, to lower ALT value, and to improve patients' immune and liver functions [Y. Liu, "Therapeutic effect of oral solution from Astragalus in the treatment of 70 chronic hepatitis B patients", *Jiang Su Chung Yao*, 15(12), 38 (1994)]. Furthermore, fractionated AM has been shown to have immunopotentiating activity, as discussed earlier. These suggest that PAGC may be used as a biological response modifier for hepatitis B carriers and to protect liver cells.

E. Vaccine adjuvant for Hepatitis B patients

Polysaccharide prepared from *Polyporus umbellatus* has been used as an adjuvant to hepatitis B vaccine in treating chronic hepatitis B patients. It has been shown to have statistically significant sero-negative conversion of hepatitis B e antigen (HBeAg) and disappearance of hepatitis B viral DNA (HBV-DNA) [S. M. Wu et al., "The therapeutic observation on the combined Polyporus polysaccharide with hepatitis B vaccine in the treatment of chronic hepatitis B". *J. Chin. Infectious Disease*, 13(3), 187-189 (1995); H. Z. Shu et al., "The therapeutic observation on the Polyporus polysaccharide with large dose of hepatitis B vaccine in the treatment of 64 cases of chronic hepatitis B patients", *Med. J. NDFSC*, 6(4), 211-212 (1996)]. Extracts prepared from AM also has been reported to have sero-negative conversion of HBeAg and anti hepatitis B core antigen (anti HBc), and elimination of HBV-DNA in hepatitis B patients [C. K. Liu et al, "Clinical and experimental studies on effects of chronic hepatitis B treated with Astragali composita", *Chung Kuo Chung Hsi I Chieh Ho Tsa Chih*, 16(7), 394-397 (1996); P. L. Chen et al., "Polysaccharide from Astragalus in treating 33 cases of chronic active hepatitis B patients", *New Drugs Clin. Remedies*, 11(2), 75-76 (1991)]. These studies and

the results discussed above suggest that PAGC may be useful as a hepatitis B vaccine adjuvant for hepatitis B patients.

Treatment of withdrawal symptoms for narcotic drug rehabilitation

When patients are removed from narcotics, they suffer from symptoms of addiction withdrawal. The attributes of withdrawal in traditional Chinese medicine (TCM) terms are manifestation of qi (energy) deficiency. Based on Chinese physicians' observations that it would speed up rehabilitation if the qi could be fortified, one of the indications for PAGC in the clinical trial of Example 9 is to improve the quality-of-life of cancer patients after chemotherapy. The quality-of-life was assessed by improvement on symptoms associated with chemotherapy; these include lassitude and fatigue, malaise, sweating, shortness of breath and lack of appetite. These symptoms correspond to "qi deficiency syndrome", which is also associated with narcotic withdrawal according to TCM physicians. This suggests that PAGC is able to fortify patients' qi and has pharmaceutical potential for treating addiction withdrawal from narcotics.

Prevention and treatment of emesis in cancer patients on chemotherapy or radiation therapy

Nausea and vomiting is a common side effect of chemotherapy and/or radiation therapy. Although there are many improvements with chemotherapy or radiation therapy, a significant number of patients still experience emesis, and efforts to reduce this side effect of treatment must continue. There are many antiemetic drugs (such as serotonin receptor antagonists, corticosteroids, and dopamine receptor antagonists) available to prevent these side effects, however, they have their own side effects. Symptoms that have been associated with these drugs are light headache, constipation, trouble sleeping, restlessness, involuntary movements of the muscles and tongue, and sedation. Although the neuropharmacologic basis of emesis is still incompletely understood, the goals related to the complete control of emesis include providing care that is convenient for the patient, treatment that reduces hospitalization and time in the ambulatory setting, and therapy that enhances the patient's quality-of-life. The clinical trial in the PRC has demonstrated that PAGC is beneficial to patients after chemotherapy, especially in

improving patients' quality-of-life. Observations from the investigators and patients all support that PAGC can really improve the overall well-being of the patients, and this suggests that PAGC may have a role in preventing and treating emesis after chemotherapy.

Reduction in or replacement of the use of erythropoietin for kidney dialysis patients

EPOGEN (epoetin alfa, erythropoietin) was approved in 1989 for treatment of the anemia associated with chronic renal failure in patients receiving dialysis. This provides a means for these patients to lead more active, productive lives. Prior to the availability of EPOGEN, 90% of all dialysis patients suffered from anemia, leaving them fatigued and exhausted and impairing their ability to work. Today, most patients undergoing dialysis receive EPOGEN as part of their treatment regimen to elevate and maintain red blood cell levels. In clinical trials, the most frequently reported adverse events with EPOGEN were hypertension, headache, seizures, nausea/vomiting, and clotted vascular access; and it is recommended to reduce the dose of EPOGEN if these side effects occur. The studies of PAGC indicated that it could promote the recovery of peripheral red blood cell counts in sublethally irradiated mice as shown in Example 6. AGPC administered in the same manner in a 20-day study at doses of 100 and 250 mg/Kg promoted both red blood cell and platelet recovery with improvement over control in irradiated mice at all points post-irradiation; demonstrating the same benefit in this model as that given by PAGC. In addition, as demonstrated in Example 7, PAGC increased the numbers of circulating BFU-E when administrated as a single agent and synergized with G-CSF to increase the numbers of circulating BFU-E in normal mice. Furthermore, PAGC-treated mice showed a significant increase of TER-119$^+$ cells in peripheral blood of both normal and cyclophosphamide-treated mice. The TER-119 antigen is expressed on erythroid cells from the early erythroblast through mature erythrocyte stages. An increase in the number of TER-119$^+$ cells indicates that PAGC could stimulate the differentiation, proliferation and maturation of the erythroid lineage in the bone marrow, and the mobilization of these cells to the peripheral blood. All these findings suggest that PAGC can promote the production and maturation of red blood cells in the mice studied. Since the use of PAGC in the clinical trials was safe and no clinically significant adverse

events were reported, it is therefore suggested that PAGC may be able to reduce or even replace the use of EPOGEN for treatment of anemia in kidney dialysis patients.

AGPC administered in a 20-day study at doses of 100 and 250 mg/Kg in the same manner as PAGC, following the procedures of Example 6, promoted both red blood cell and platelet recovery with improvement over control in irradiated mice at all points post-irradiation; demonstrating the same benefit in this model as that given by PAGC; and suggesting, in conclusion, when taken with the other PAGC data discussed in this application, that AGPC has the same therapeutic benefits as PAGC, and is therefore suitable for the same utilities and pharmaceutical indications as PAGC.

Pharmaceutical formulations and administration

In general, the purified arabinogalactan composition of the first aspect of this invention or the arabinogalactan protein composition of the second aspect of this invention will be administered in therapeutically effective amounts by intravenous injection, either singly or in conjunction with of at least one other therapeutic agent, especially a therapeutic agent capable of stimulating hematopoiesis. A therapeutically effective amount may vary widely depending on the disease, its severity, the age and relative health of the animal being treated, and other factors. For stimulating hematopoiesis, inducing the proliferation or maturation of megakaryocytes, stimulating the production of IL-1β, IL-6, TNF-α, IFN-γ, GM-CSF, or G-CSF, stimulating the production or action of neutrophils, treating neutropenia, anemia, or thrombocytopenia, or accelerating recovery from exposure (e.g. accidental or non-therapeutic exposure, as well as therapeutic exposure) to cytotoxic agents or radiation, a therapeutically effective amount of the purified arabinogalactan composition of the first aspect of this invention ranges about between 50 and 1000 mg/day, particularly about between 100 and 500 mg/day, especially about 250 mg/day, for a human of average body mass. For treating cachexia, emesis, or drug withdrawal symptoms, or modifying biological responses or protecting hepatic cells in hepatitis B, a similar amount will be therapeutically effective. Because the arabinogalactan protein composition of this invention has a higher content of the arabinogalactan protein itself, it is expected to be more potent, and will have a correspondingly lower therapeutically effective amount,

such as about between 10% and 50% of the therapeutically effective amount of the purified arabinogalactan composition. A person of ordinary skill in the art will be able without undue experimentation, having regard to that skill and this disclosure, to determine a therapeutically effective amount of the compositions of this invention for a given disease.

In general, the purified arabinogalactan composition of the first aspect of this invention or the arabinogalactan protein composition of the second aspect of this invention will be administered as pharmaceutical formulations by intravenous injection. The formulation will comprise the purified arabinogalactan composition of the first aspect of this invention or the arabinogalactan protein composition of the second aspect of this invention in combination with an aqueous intravenously injectable excipient. Suitable aqueous intravenously injectable excipients are well known to persons of ordinary skill in the art, and they, and the methods of formulating the formulations, may be found in such standard references as Alfonso AR: Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton PA, 1985. Suitable aqueous intravenously injectable excipients include water, aqueous saline solution, aqueous dextrose solution, and the like.

Typically, when administered as a hematopoietic agent, the purified arabinogalactan composition of the first aspect of this invention or the arabinogalactan protein composition of the second aspect of this invention will be administered by intravenous injection, especially by continuous intravenous infusion over a period of a few minutes to an hour or more, such as around fifteen minutes. The amount of a compound of this invention in the composition may vary widely depending on the type of composition, size of a unit dosage, kind of excipients, and other factors well known to those of ordinary skill in the art. In general, the final composition may comprise from 0.001 percent by weight (%w) to 10 %w of the compound of this invention, preferably 0.01 %w to 1 %w, with the remainder being the excipient or excipients.

The purified arabinogalactan composition of the first aspect of this invention and the arabinogalactan protein composition of the second aspect of this invention may optionally be administered in conjunction with at least one other therapeutic agent for the

disease state being treated, especially another agent capable of stimulating hematopoiesis such as, for example, erythropoietin, thrombopoietin, granulocyte colony stimulating factor (G-CSF), IL-3, and the like.

Examples

The following non-limiting examples illustrate the invention. All purified arabinogalactan compositions and arabinogalactan protein compositions were characterized by size exclusion chromatography.

The size exclusion chromatography method was as follows:
A Shimadzu HPLC system, equipped with an SCL-10A system controller, LC-10AD pump, DGU-4A degasser, RID-6A refractive index detector, and SPD-10AV UV detector, and using GS-701 and GS-620 columns (Shodex Asahipak, 7.6 x 500 mm) equilibrated with 0.2 *N* sodium chloride. The sample amount loaded was 80 μg (40 μL of sample solution at 2 mg/mL in water), and samples were eluted at 1 mL/min. Pullulan standards with different average molecular weight were used to prepare a calibration curve; and the molecular weights were determined from the calibration curve. The weight average molecular weight ($Mw = \sum(A_i M_i) / \sum A_i$), number average molecular weight ($Mn = \sum A_i / \sum(A_i/M_i)$), and polydispersity (Mw / Mn) of a sample was determined by statistical calculation using the Shimadzu SEC software, Version 2.4.

The sugar content and composition of the PAGC and AGPC of this invention was determined by GLC analysis of the trimethylsilyl methyl glycoside derivatives. In this method, the polysaccharide was first methanolyzed in methanolic HCl, followed by a trimethylsilyl (TMS) derivatization to generate volatile monosaccharide derivatives. After a clean-up, the derivatives were analyzed by Gas Liquid Chromatography (GLC) using a DB-1 column with a Flame Ionization Detector (FID). An internal standard, *myo*-inositol, was derivatized and analyzed together with the composition sample to quantitate the sugar content and composition.

Hydroxyproline content was determined by a colorimetric assay. The sample was first hydrolyzed with hydrochloric acid, then treated with sodium hypobromite (a solution of bromine in sodium hydroxide), hydrochloric acid, and dimethylamino-

benzaldehyde. The optical density of the final solution was measured on a colorimeter, with the hydroxyproline content determined from a calibration curve made from hydroxyproline of various concentrations prepared in the same manner.

Example 1. Preparation of the arabinogalactan composition

Step A. "Drink chip" processing

Dried *Astragalus membranaceus* roots, 300 Kg, were processed into drink chips by removing any contaminated parts, sterile washing and scrubbing with ultrafiltered water, soaking in 70% ethanol overnight, cutting into chips with a thickness of 3 – 5 mm, and sterile oven drying at 60 – 70 °C. These dried "drink chips" have a loss on drying of <15%.

Step B. Crude arabinogalactan composition extraction

"Drink chips", 200 Kg, produced in Step A, were extracted three times with UF water (deionized water ultrafiltered through a 10K MWCO UF system) at 100 °C, each time for three hours. The pooled water extract was concentrated with a concentrator at 60 – 70 °C to 200 L under reduced pressure. Ethanol, 95%, was added to the concentrate to give a final ethanol concentration of 70%, with stirring at room temperature for fifteen minutes, to precipitate polysaccharides. The supernatant was decanted and the precipitate was washed three times with 95% ethanol. The precipitate was re-suspended in UF water to a concentration of 18 – 20%, as measured by a refractometer, and 95% ethanol was added to give a final ethanol concentration of 35%. The ethanol suspension was centrifuged, the precipitate was discarded, and 95% ethanol was added to the supernatant with stirring to give a final ethanol concentration of 70%. The precipitate was collected, re-dissolved in UF water, and spray dried to generate the crude arabinogalactan composition.

The crude arabinogalactan composition was a light-yellow powder, soluble in water at 200 mg/mL, and had a loss on drying of <15%. The endotoxin content was <0.3 EU/mg.

Step C. Purification of the arabinogalactan composition

The crude arabinogalactan composition, 3.5 Kg, from Step B was dissolved in UF water to a concentration of 2% (volume of 175 L). The solution was filtered through a 5K MWCO UF system to a final volume of 35 – 40 L. The concentrated solution was adjusted to a 20 m*M* NaOAc buffer concentration at pH 5.2 by the addition of 1.0 *M* NaOAc buffer, pH 5.2. The solution was loaded onto an SP Sepharose cation exchange column (20 L volume, 30 cm bed height) and eluted with 20 m*M* NaOAc, collecting 2.5 – 3.0 bed volumes of the eluate. The collected eluate was loaded onto a Q Sepharose anion exchange column with the same resin volume and bed height as the SP column and eluted with 20 m*M* NaOAc, collecting 3 – 3.5 bed volumes of the eluate. The collected eluate from the Q Sepharose column was filtered through a 0.1 μm filter and then ultrafiltered with an 8K MWCO UF system. The retentate was condensed to 20 – 26% by a concentrating system at 50 – 60 °C and precipitated by addition of anhydrous ethanol to a final ethanol concentration of 80 – 90%. The precipitate was washed three times with anhydrous ethanol and dried in a vacuum oven at 60 – 70 °C to give the purified arabinogalactan composition.

The purified arabinogalactan composition was a white powder, soluble in water, saline, and 5% glucose at 20 mg/mL, and having a water content of ≤ 6.0%. The composition contained ≤ 2.0% of ash, ≤ 10 ppm of heavy metals, and ≤ 0.1 EU/mg of endotoxin. An aqueous solution of the composition had a pH between 4.5 and 6.5. The composition had a sugar content of ≤ 85% (determined by the phenol-H_2SO_4 method with glucose as standard), and an Ara:Gal ratio of ≥ 1.5:1 (determined by GLC of the TMS-methylglycoside derivatives of the composition).

Example 2. Preparation of the arabinogalactan protein composition

The collected eluate from the Q Sepharose column of Step C of Example 1 was ultrafiltered with a 100K MWCO UF system. The retentate from the ultrafiltration was further concentrated, and precipitated by addition of anhydrous ethanol to a final ethanol concentration of 80 – 90%. The precipitate was washed three times with anhydrous

ethanol and dried in a vacuum oven at 60 – 70 °C to give the purified arabinogalactan composition.

The arabinogalactan protein composition was a white powder, soluble in water, saline, and 5% glucose at 20 mg/mL, with an aqueous solution having a pH between 4.5 and 6.5. The composition contained ≤ 0.5 EU/mg of endotoxin, and ≤ 10 ppm of heavy metals. It had an Ara:Gal ratio of ≥ 2:1 and contained more than 0.2% hydroxyproline. The weight average molecular weight of the composition was ≥ 100 kiloDaltons.

Example 3. Production of cytokines from activated human peripheral blood mononuclear cells

Human peripheral blood mononuclear cells (PBMC) were prepared using the method of Boyum [A. Boyum, "Isolation of mononuclear cells and granulocytes from human blood. . . . ", *Scan. J. Lab. Invest.*, 97, 77-89 (1968)]. Human blood buffy coat samples, approximately 25 mL/donor, were obtained from the Stanford University Medical Center Blood Bank. Using sterile techniques, each buffy coat samples was gently resuspended in a total volume of 100 mL with the addition of calcium- and magnesium-free Hank's balanced salt solution (HBSS, Gibco) at room temperature. A volume of 25 mL of the cell suspension was then layered onto 15 mL of Ficoll-Paque (Pharmacia LKB Biotechnology, Inc.) in a 50 mL conical centrifuge tube, and the tube was centrifuged in a Beckman GPR tabletop centrifuge (GH-3.7 rotor) at 400 × g for 30 minutes at 15 °C. Following centrifugation, the PBMC suspension at the interface was transferred to a new 50 mL tube, resuspended in a total volume of 45 mL HBSS, and centrifuged at 354 × g for 10 minutes at 15 °C. The supernatant was discarded, the cell pellets was resuspended to a total of 45 mL with HBSS, and centrifuged again at 265 × g for 10 minutes at 15 °C. The cell pellet was resuspended in 10 ml of X-Vivo tissue culture medium (Bio Whittaker, MD) and counted using a hemocytometer. Polystyrene tubes (Falcon # 2057, Becton Dickinson) and PBMC from 2 different donors were used in the following experiment. PBMC suspensions were diluted to 4×10^6/mL; 1 mL was incubated in the presence of 0.5 mL phytohemagglutinin P (PHA-P, Pharmacia 27-3707-01) at a final concentration of 3 µg/mL together with 0.5 mL of a solution of one of the compositions of this invention at various concentrations. The total volume per

tube was 2 mL. Another aliquot of cells treated with PHA alone served as control. After 24 hours incubation at 37 °C in a incubator with 7% CO_2, the tubes were centrifuged in a Beckman GPR tabletop centrifuge (GH-3.7 rotor) at 1600 × g for 10 minutes at 15 °C, and the supernatants were collected and stored at -70 °C prior to assay. Cytokine measurements were carried out using commercially available ELISA assay kits for human cytokines IL-1β, IL-6, TNF-α, GM-CSF and G-CSF (R&D Systems, MN) and human IFN-γ (Endogen, MA) in accordance with the manufacturer's protocols. Optical density was determined using a microplate reader (Thermo max, Molecular Devices, CA). Results were calculated using the software provided with the microplate reader and expressed as pg/mL of cytokine produced in the supernatants. All results were expressed as a ratio of sample to control (S/C) where S is the amount of cytokine produced in PBMC stimulated with PHA plus test sample and C is the amount of cytokine produced in PBMC stimulated with PHA alone. The following table shows that PAGC increased cytokine production by activated human PBMC.

PAGC, μg/mL	ELISA (S/C)					
	IL-1β	IL-6	TNF-α	IFN-γ	GM-CSF	G-CSF
25	9.1	11.8	4.9	3.1	6.5	49.9
10	4.3	5.9	2.3	1.2	2.1	16.5

Example 4. Proliferation/maturation of bone marrow megakaryocytes

C3H/HeJ mice, male, 9-14 weeks old, were used for the experiment. The liquid culture assay for megakaryocyte maturation is an *in vitro* assay for studying the proliferation and/or maturation of bone marrow megakaryocytes (the progenitors of peripheral blood platelets). A detailed protocol is given in S. A. Burstein, "Interleukin 3 promotes maturation of murine megakaryocytes *in vitro*.", *Blood Cells*, 11, 469-479 (1986). Normal mouse bone marrow mononuclear cells were isolated and suspended in medium containing 0.5 m*M* diisopropylflourophosphate (DFP, Sigma, St. Louis, MO) at room temperature for 20 minutes to inactivate endogenous acetylcholinesterase (AchE). The cells were washed, resuspended, and placed in a plastic tissue culture flask at

4×10^6/mL in 15% FCS-IMDM (Gibco BRL, Gaithersburg, MD) to remove stromal cells and macrophages by their adherence to the flask. The flask containing resuspended bone marrow cells was incubated at 37 °C, 5% CO_2 for 1.5 hours. Non-adherent cells were collected and resuspended at 1×10^6/mL in 1% Nutridoma SP (Boehringer Mannheim, Indianapolis, IN)-IMDM for assay. The cells were added to 96-well, U-bottom tissue culture plates, 10^5 cells/well in a final volume of 0.2 mL tissue culture medium containing various concentrations of PAGC with and without 50 pg/mL of murine recombinant IL-3 (R & D Systems, Minneapolis, MN). The cultures were incubated at 37 °C, 5% CO_2 for seven days. The activity of PAGC on the megakaryocytes was determined by assaying the increase in AChE activity, a relatively specific marker for rodent megakaryocytes, where an increase is taken as an index of proliferation or maturation of megakaryocytes. After seven days of culture, the plates were centrifuged and the supernatant was discarded. Solution I (0.2% Triton X-100, 1 m*M* EDTA, 0.12 m*M* NaCl, 50 m*M* HEPES, pH 7.5), 0.2 mL, and 20 µL 6.27 m*M* acetylthiocholine iodide (Sigma, St. Louis, MO) were added to each well (the final concentration of acetylthiocholine iodide is 0.57 m*M*). The plates were incubated at 37 °C for four hours, then 10 µL of reaction mixture from each well was transferred into the wells of a 96 well black plate (Labsystems, Helsinki, Finland), and 10 µL of 0.4 m*M* coumarinphenylmaleimide (CPM, Molecular Probes Inc., Eugene, OR) in DMSO was then added, followed by 0.2 mL of Solution II (5 m*M* sodium acetate, 1 m*M* EDTA, 0.2% Triton X-100, pH 5.0). The plate was mixed by gentle shaking. The emitted fluorescence was measured in a fluorimeter (Fluoroskan II, Labsystems, Helsinki, Finland) with an excitation filter of 390 nm and an emission filter of 460 nm. The readings from the fluorimeter are directly proportional to the production of AChE from megakaryocytes in the culture. PAGC at concentrations between 12.5 µg/mL and 400 µg/mL (with a peak at 200 µg/mL) increased the number of megakaryocytes in the culture, and that PAGC at concentrations between 12.5 µg/mL and 400 µg/mL (with a peak at 200 µg/mL) synergized with 50 pg/mL IL-3 to greatly increase the number of megakaryocytes in the culture (more than doubling the number of megakaryocytes at concentrations between 50 and 200 µg/mL).

Example 5. Recovery of GM-CFC progenitor cells in fluorouracil-treated mice

Female BALB/c mice, 8-10 weeks old, were treated with 150 mg/Kg fluorouracil (Sigma) intraperitoneally on day 0, dosed subcutaneously with saline, various concentrations of PAGC or 100 µg/Kg recombinant human G-CSF (Neupogen, Amgen) on days 1-3, and sacrificed on day 4. Femoral bone marrow was harvested, counted, and 5×10^4 leukocytes were plated in 35 mm Petri dishes in triplicate 1 mL cultures in complete methylcellulose medium with pokeweed mitogen spleen cell conditioned medium as a source of colony stimulating factors (StemCell Technologies Inc). Colonies of greater than 50 cells were scored using a Nikon Diaphot microscope (30-60×) after 6-7 days of incubation at 37°C, with the following table showing that PAGC augmented GM-CFC progenitor recovery in fluorouracil-treated mice.

Treatment	Saline	PAGC, 50 mg/Kg	PAGC, 100 mg/Kg	PAGC, 200 mg/Kg	G-CSF, 100 µg/Kg
GM-CFC/femur	601	608	1075	2020	1196

Example 6. Recovery of peripheral blood in sublethally irradiated mice

Female BALB/c mice with an average body weight of 20 grams; 9-14 weeks old, were used for the study. Five days before each experiment, Neomycin (Sigma, St. Louis, MO), 40 mg/L, was added to non-acidified drinking water. Mice were randomly assigned to control or treated group, 6 mice per group, and were irradiated were irradiated with 4.25 Gy of X-rays (250 KVP, 0.35 mm Cu filter, Philips, Germany) on day 0. Following this dose of irradiation, the peripheral blood leukocyte and platelet counts are significantly lower than that of normal mice, and the erythrocyte count is moderately lower. PAGC was given by subcutaneous injection at 300 and 100 mg/Kg. Treatments were given each for the first 5 days (from day 0 to day 4), and then 3 times a week for the next 3 weeks, with the first dose given 4-5 hours after irradiation. A total of 14 doses of PAGC were administered. The control group was given 0.1 mL saline subcutaneously. Mice were bled through the tail veins twice/week during the experiment; blood samples were collected into EDTA-coated tubes (Sarstedt, Germany); and peripheral blood white blood cells, platelets, red blood cells, and hemoglobin were analyzed in a Serono 9010+

cell counter (Serono Baker Diagnostics Inc., Allentown, PA). PAGC at doses of 100 and 300 mg/Kg promoted white blood cell recovery in irradiated mice with statistically significant improvement ($p<0.01$) over control from days 17 through 30 (end of the trial) post-irradiation; promoted platelet recovery in irradiated mice with statistically significant improvement ($p<0.05$, generally $p<0.01$) over control from days 14 through 25 post-irradiation; and promoted red blood cell recovery in irradiated mice with statistically significant improvement ($p<0.05$, generally $p<0.01$) over control from days 14 through 25 post-irradiation. AGPC administered in the same manner in a 20-day study at doses of 100 and 250 mg/Kg promoted both red blood cell and platelet recovery with improvement over control in irradiated mice at all points post-irradiation; demonstrating the same benefit in this model as that given by PAGC.

Example 7. Mobilization of peripheral blood progenitor cells with PAGC alone or in combination with granulocyte colony-stimulating factor (G-CSF)

Female BALB/c mice, 8-10 weeks old, were given acidified water and food ad libitum. Normal mice were treated for seven days, once per day, as follows: saline (200μL, subcutaneously), PAGC(100 mg/Kg, subcutaneously), G-CSF (100 μg/Kg, subcutaneously), or PAGC+G-CSF (100 mg/Kg PAGC plus 100 μg/Kg G-CSF, subcutaneously). Injection volumes were approximately 200 μL per mouse. Each group consisted of 5 animals each. After seven days of treatment as described above, on day 8 mice were treated intraperitoneally with 20 units of heparin (Elkins-Sinn, Inc., Cherry Hill, NJ), sacrificed by CO_2 inhalation 30 minutes post-heparin treatment, and peripheral blood was collected by cardiac puncture. Peripheral blood mononuclear cells were isolated by density centrifugation using Ficoll-Paque (Pharmacia Biotech AP, Uppsala, Sweden), washed twice in phosphate buffered saline, resuspended in medium and counted using a hemocytometer. Between 2.5×10^4 and 1×10^5 peripheral blood mononuclear cells were plated in 35 mm Petri dishes in triplicate 1 mL cultures in complete erythropoietin-containing methylcellulose medium with recombinant IL-3 + IL-6 + stem cell factor (StemCell Technologies Inc., Vancouver, B.C.). Colonies of greater than 50 cells were scored using a Nikon Diaphot microscope (30-150×) after 7-14 days of incubation at 37°C. Both granulocyte macrophage colony forming cells (GM-CFC) and burst forming units – erythroid (BFU-E) were scored. The following

table shows that PAGC synergized with G-CSF to increase the number of circulating GM-CFC and BFU-E in normal mice.

	Saline	PAGC, 100 mg/Kg	G-CSF, 100 µg/Kg	PAGC, 100 mg/Kg + G-CSF, 100 µg/Kg
GM-CFC, 100/mL	0.8	4.6	30	64
BFU-E, 100/mL	1.0	3.1	4.5	9.5

In a similar experiment where the mice were treated intravenously with 200 mg/Kg cyclophosphamide and then dosed with saline or PAGC at 100 or 300 mg/Kg for eleven days, the PBMC collected and suspended at 2×10^7 cells/mL, stained with fluorescein isothiocyanate-anti-CD34 and PE-lineage markers (CD3, CD4, CD6, CD19, CD11b, GR-1, CD41, and Ter-119) (Pharmingen, San Diego, CA) and analyzed by flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA), it was seen that PAGC increased the mobilization of $CD34^+Lin^-$ cells into the peripheral blood.

Example 8. Recovery of body weight by administration of PAGC in fluorouracil- or cyclophosphamide-treated mice

Female BALB/c mice, 8-10 weeks old, were used in this study. Ten mice each were randomized to one of the 5 groups, normal control, cyclophosphamide (CY)-treated, fluorouracil (FU)-treated, CY+PAGC-treated or FU+PAGC-treated. For the normal control group, mice were injected intraperitoneally with saline on day 0 and subcutaneously with saline from day 1 through day 12. The CY-treated or CY+PAGC-treated group were given 200 mg/Kg cyclophosphamide intraperitoneally on day 0, and then treated subcutaneously with saline or 200 mg/Kg PAGC from day 1 through day 12. The FU-treated or FU+PAGC-treated group were given 150 mg/Kg fluorouracil intraperitoneally on day 0, and then treated subcutaneously with saline or 200 mg/Kg PAGC from day 1 through day 12. The mice were weighed on day 0 and then every other day through day 12. The results show that mice treated with CY+PAGC and FU+PAGC lose less weight and regain weight faster than those treated with CY or FU alone.

Example 9. Human clinical trials with PAGC in the Peoples' Republic of China

Human clinical trials with PAGC in the Peoples' Republic of China were conducted in accordance with the PRC Ministry of Public Health GCP standards.

In a Phase I clinical trial, PAGC diluted in normal saline was administered intravenously to 32 normal volunteers by continuous infusion for seven days. There were no clinically significant adverse reactions associated with the administration of PAGC at up to three times the intended clinical dosage (250 mg/day).

A Phase II trial was conducted to evaluate the efficacy of PAGC in alleviating chemotherapy-induced leukopenia ($<4.0 \times 10^9$ WBC/L) in patients with lung, gastroenteric, or breast cancer. Six centers were involved in this study and had 487 patients entered into the study: of these, 328 patients were in the PAGC group, 84 in the G-CSF group, and 75 in the no-treatment group. When a patient's WBC count was less than 4.0×10^9/L anytime during the fourteen days after receiving chemotherapy, the patient was assigned to one of the three groups. To patients in the first group, 250 mg PAGC dissolved in 500 mL of normal saline was administered intravenously once daily for seven days. These patients were monitored during the period of PAGC administration and for another seven days. Patients in the G-CSF group received 75 μg G-CSF subcutaneously once daily for five days, and were monitored during the period of administration and for additional nine days. No blood tonifying medications were used after the chemotherapy in the third (no-treatment) group and the patients were monitored for fourteen days. Thus all three groups were observed, and their WBC, RBC, and platelet counts taken, for fourteen days after the beginning of treatment. Patients were also monitored for their quality-of-life assessed by improvement on the symptoms associated with chemotherapy and their Karnofsky Performance Index. The symptoms associated with chemotherapy included lassitude and fatigue, malaise, sweating, shortness of breath, and lack of appetite, and were assigned a score by a qualified Traditional Chinese Medicine physician. In each category the assignment was: 0 point - no symptoms, 1 point - mild symptoms, 2 points - moderate symptoms and 3 points - severe symptoms. The most severe case thus would score 15 points. The Karnofsky Performance Index is a generic health status measurement according to WHO. FIG. 1

shows the mean white blood cell counts versus days after chemotherapy; FIG. 2 shows the total symptom scores versus days after chemotherapy; FIG. 3 shows the scores on the Karnofsky Performance Index (PAGC vs. G-CSF: U=18.36, p<0.01; PAGC vs. no treatment: U=15.62, p<0.0001); and the table below shows the mean platelet count ± standard deviation versus days after chemotherapy; all indicating that PAGC at 250 mg for seven days promotes WBC recovery and platelet recovery, improves chemotherapy-induced symptoms, and improves the Karnofsky Performance Index of chemotherapy patients, generally with a statistically significant improvement over no treatment, and frequently with a statistically significant improvement over G-CSF at 75 μg for five days.

Platelets, 10^9/L, in PAGC-treated patients (n = 54)				
Day 0	Day 4	Day 7	Day 10	Day 14
65 ± 21	87 ± 61*	118 ± 94**	149 ± 107**	178 ± 111**
* p<0.05, ** p<0.001				

While this invention has been described in conjunction with specific embodiments and examples, it will be apparent to a person of ordinary skill in the art, having regard to this disclosure, that equivalents of the specifically disclosed materials and techniques will also be applicable to this invention; and such equivalents are intended to be included within the following claims.

WHAT IS CLAIMED IS:

1. A purified arabinogalactan composition isolated from *Astragalus membranaceus*, especially from the roots of *Astragalus membranaceus*.

2. The purified arabinogalactan composition of Claim 1 where the *Astragalus membranaceus* is *A. membranaceus* Bge. var. *mongholicus* (Bge.) Hsiao or *A. membranaceus* (Fisch.) Bge.

3. The purified arabinogalactan composition of Claim 1 or 2 that is isolated from *Astragalus membranaceus* plants grown in Inner Mongolia or Shanxi province, Peoples' Republic of China, especially the former.

4. The purified arabinogalactan composition of any of Claims 1 to 3 where the *Astragalus membranaceus* plants are two-year old *Astragalus membranaceus* plants.

5. The purified arabinogalactan composition of any of Claims 1 to 4 having a weight average molecular weight of 20 kiloDaltons to 60 kiloDaltons.

6. The purified arabinogalactan composition of any of Claims 1 to 5 having an arabinose/galactose ratio of at least 1.5.

7. The purified arabinogalactan composition of any of Claims 1 to 6 having an endotoxin content of not more than 1.0 EU/mg.

8. An arabinogalactan protein composition, having a weight average molecular weight of at least 100 kiloDaltons, isolated from a purified arabinogalactan composition of any of Claims 1 to 7.

9. An aqueous intravenously injectable arabinogalactan formulation comprising:

(a) a therapeutically effective amount of the purified arabinogalactan composition of any of Claims 1 to 7 or the arabinogalactan protein composition of Claim 8; and

(b) an aqueous intravenously injectable excipient.

10. A method of treating a disease state in a mammal capable of treatment by administration of the purified arabinogalactan composition of any of Claims 1 to 7 or the arabinogalactan protein composition of Claim 8, comprising intravenously administering to the mammal an effective amount of the purified arabinogalactan composition of any of Claims 1 to 7, the arabinogalactan protein composition of Claim 8, or the aqueous intravenously injectable arabinogalactan formulation of Claim 9.

11. The method of Claim 10 where the method is a method of stimulating hematopoiesis, inducing the proliferation or maturation of megakaryocytes, stimulating the production of IL-1β, IL-6, TNF-α, IFN-γ, GM-CSF, or G-CSF, stimulating the production or action of neutrophils, treating neutropenia, anemia, or thrombocytopenia, accelerating recovery from exposure to cytotoxic agents or radiation, treating cachexia, emesis, or drug withdrawal symptoms, or modifying biological responses or protecting hepatic cells in hepatitis B.

12. The method of Claim 11 where the method is a method of stimulating hematopoiesis, inducing the proliferation or maturation of megakaryocytes, stimulating the production of IL-1β, IL-6, TNF-α, IFN-γ, GM-CSF, or G-CSF, stimulating the production or action of neutrophils, or treating neutropenia, anemia, or thrombocytopenia.

13. The method of any one of Claims 10 to 12 where the mammal is a human.

14. The method of Claim 12 or 13 where the mammal is suffering from bone marrow suppression.

15. The method of Claim 14 where the bone marrow suppression is the result of cancer chemotherapy or radiation therapy.

16. The method of any one of Claims 10 to 15 further comprising the administration of at least one other therapeutic agent.

17. The method of Claim 15 where the at least one other therapeutic agent is a therapeutic agent capable of stimulating hematopoiesis.

18. The method of Claim 17 where the at least one other therapeutic agent is selected from erythropoietin, thrombopoietin, granulocyte colony stimulating factor, or IL-3.

19. A method of producing the purified arabinogalactan composition of Claim 1, comprising:

(a) extracting from *Astragalus membranaceus* an aqueous extract containing an arabinogalactan composition;

(b) adding to the aqueous extract from step (a) sufficient lower alkanol to precipitate the arabinogalactan composition, and isolating the precipitated arabinogalactan composition;

(c) dissolving the precipitated arabinogalactan composition from step (b) in water to form an arabinogalactan composition-containing solution;

(d) treating the arabinogalactan composition-containing solution from step (c) to remove materials having a molecular weight less than the molecular weight of the arabinogalactan composition;

(e) purifying the arabinogalactan composition-containing solution from step (d) by ion exchange chromatography; and

(f) isolating the purified injectable arabinogalactan composition from the purified arabinogalactan composition-containing solution from step (e).

20. A method of producing the arabinogalactan protein composition of Claim 8, comprising

(a) subjecting an aqueous solution of a purified arabinogalactan composition of any one of Claims 1 to 7 to ultrafiltration through an ultrafilter having a 100 kiloDalton molecular weight cutoff; and

(b) isolating the arabinogalactan protein composition from the retentate from step (a).

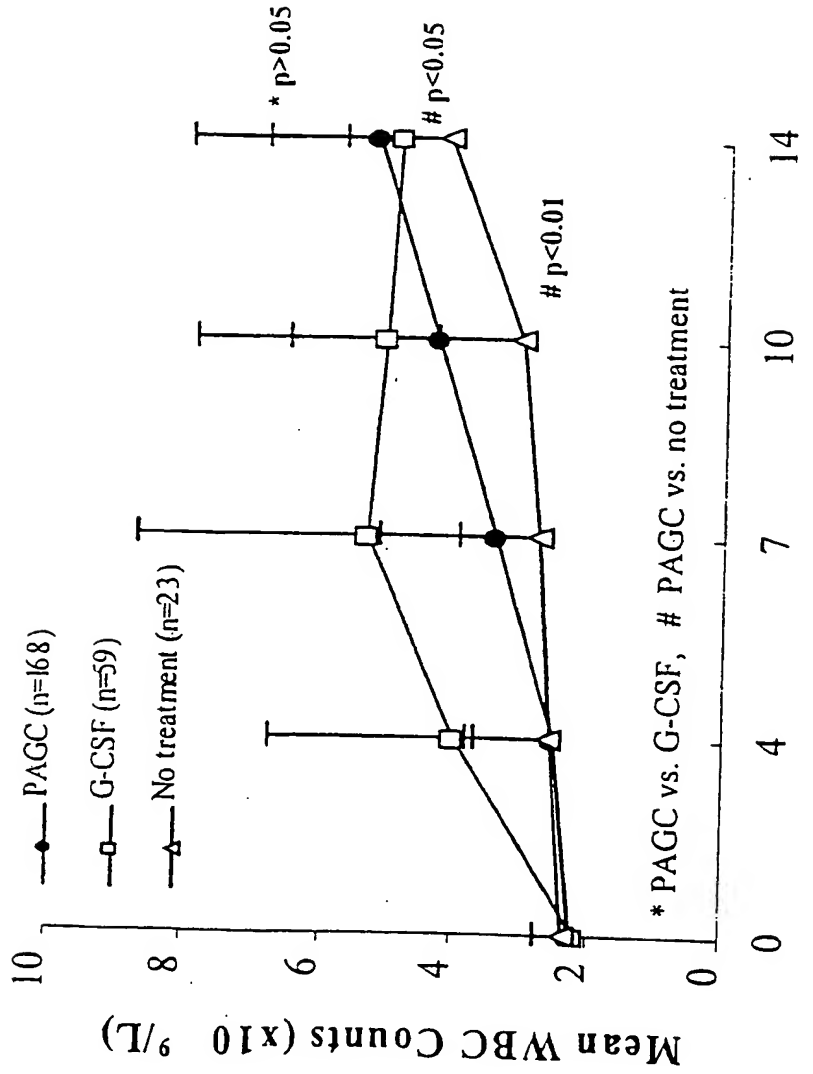
PAGC (n=168)
G-CSF (n=59)
No treatment (n=23)
* p>0.05
# p<0.05
# p<0.01
* PAGC vs. G-CSF, # PAGC vs. no treatment
Mean WBC Counts (x10 9/L)
Days
0
2
4
6
8
10
0
4
7
10
14

FIG. 1

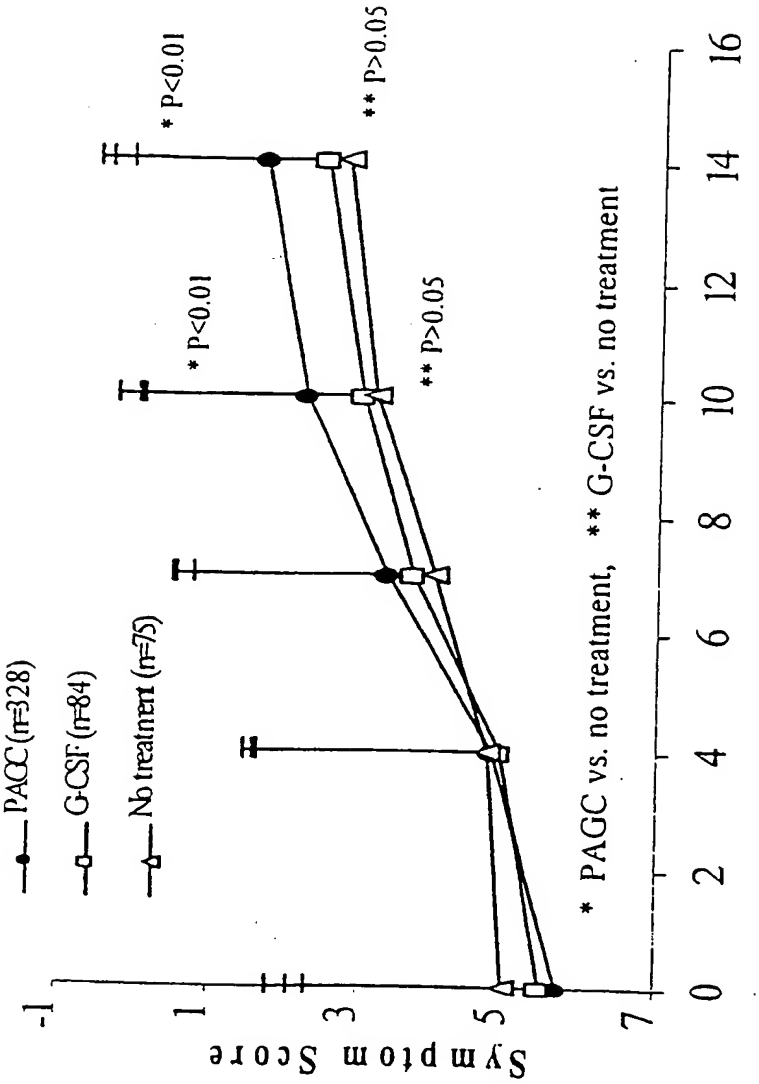
PAGC (n=328)
G-CSF (n=84)
No treatment (n=75)
* P<0.01
* P<0.01
** P>0.05
** P>0.05
* PAGC vs. no treatment, ** G-CSF vs. no treatment
Symptom Score
-1
1
3
5
7
0
2
4
6
8
10
12
14
16
Days

FIG. 2

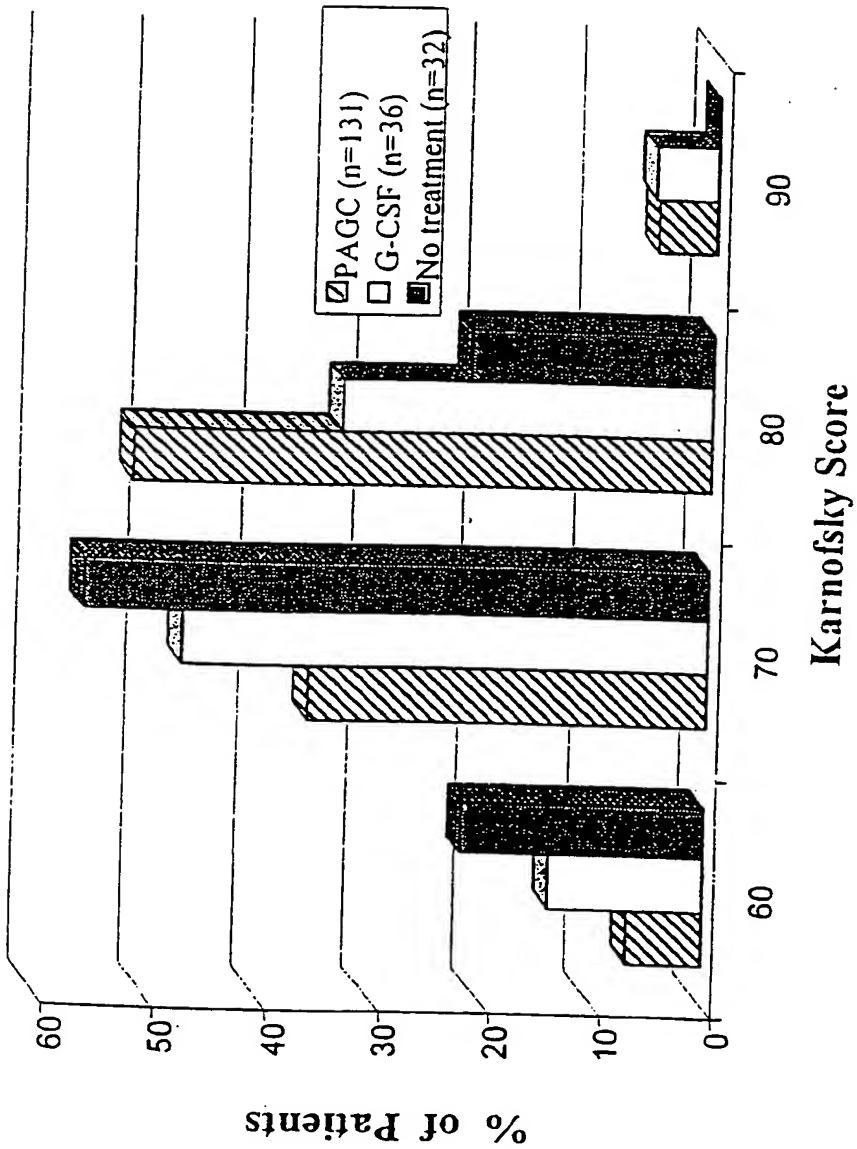
PAGC (n=131)
G-CSF (n=36)
No treatment (n=32)
% of Patients
0
10
20
30
40
50
60
60
70
80
90
Karnofsky Score

FIG. 3

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 00/18180

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C08B37/00 A61K31/715

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C08B A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DISSERTATION ABSTRACTS INTERNATIONAL, vol. 47, no. 7, 7 January 1987 (1987-01-07), page 2891 XP000942629 & MCLAUGHLIN, MICHAEL ATKINS: "Identification of Immunostimulants derived from Astragalus Membranaceus" 1986 , DISSERTATION, TEXAS A & M UNIVERSITY , COLLEGE STATION, TX, US	1-20
X Y	EP 0 441 278 A (INDENA SPA) 14 August 1991 (1991-08-14) column 1, line 24-48	1-6 11-15, 17-19

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 October 2000	08/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Radke, M

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 00/18180

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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		AT 151991 T	15-05-1997
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		CA 2105068 A	03-10-1992
		DE 578765 T	26-05-1994
		EP 0578765 A	19-01-1994
		ES 2061423 T	16-12-1994
		GR 94300022 T	29-04-1994
		JP 6506230 T	14-07-1994
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